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Differences in cytokine secretion by intestinal mononuclear cells, peripheral blood monocytes and alveolar macrophages from HIV-infected patients

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SUMMARY

Mononuclear cells of the lamina propria (LpMNC), isolated from endoscopically taken biopsies of the large bowel from AIDS patients, were analysed for their ability to secrete tumour necrosis factor-alpha (TNF-α), IL-1β and IL-6. Stimulation of LpMNC from normal controls with pokeweed mitogen (PWM) led to a time- and dose-dependent enhancement of TNF-α, IL-1β and IL-6 secretion. In contrast, PWM stimulation of LpMNC from AIDS patients resulted in only a small increase in TNF-α release. Constitutive secretion of IL-1β and IL-6 in these patients was already increased to the concentration range of stimulated cells from normal controls and could not be further increased, probably due to maximal *in vivo* stimulation. Secretion of TNF-α, IL-1β and IL-6 by peripheral blood monocytes (PBM) and alveolar macrophages from AIDS patients was elevated with or without stimulation compared with normal controls. Obviously, the regulation of TNF-α secretion is dependent on the microenvironment. Since it is known that interferon-gamma (IFN-γ) may induce the production of TNF-α, the secretion of this cytokine was examined. Release of IFN-γ was constitutively and under stimulation lowered in LpMNC from AIDS patients compared with normal controls. Addition of IFN-γ to LpMNC did not result in enhanced TNF-α secretion. Our data indicate a defective function of intestinal mononuclear cells in AIDS patients as shown by the diminished TNF-α secretion.

Keywords tumour necrosis factor IL-1 IL-6 interferon-gamma AIDS mucosal immune system alveolar macrophages

INTRODUCTION

High levels of tumour necrosis factor-alpha (TNF-α) have been observed in sera [1–3] as well as in supernatants of cultured monocytes [4,5], peripheral blood mononuclear cells (PBMC) [3,6,7] and alveolar macrophages [8–10] from AIDS patients. Several authors have measured IL-1 in HIV-infected patients with conflicting results [3,4,11,12]. TNF-α and IL-1 are mainly produced by activated mononuclear phagocytes [13,14]. Several lines of evidence show that TNF-α and IL-1 are multifunctional cytokines with a similar broad range of biological activities. As mediators, they may be involved in the induction of fever [15], in tissue repair by stimulation of fibroblast growth [16,17], in bone resorption [18,19] and in alterations of the lipid metabolism [13,20]. Both mediators are important regulators of the acute phase response in inflammation and infection [17]. TNF has a chemotactic effect on macrophages and granulocytes [21], can stimulate the phagocytic activity as well as the respiratory burst activity and the superoxide anion formation of neutrophils [22–24]. It enhances the cytotoxicity of monocytes [25] and eosinophils [26], and promotes the expression of HLA-DR antigen and IL-2 receptor on activated T cells [27]. Both cytokines induce biosynthesis and transient expression of the antigen on the surface of cultured human vascular endothelial cells recognized by monoclonal MoAb H4/18 [28]. *In vivo*, these cytokines seem to cause changes on endothelial cells which in turn affect procoagulant activity [29,30] and leucocyte adhesion [31] at the site of injury or infection. Since TNF-α and IL-1 induce expression of other cytokines and are themselves released in response to a number of cytokines [15,25,32–34], they are important factors in the complex cytokine network. IL-1 and TNF-α as well as other mediators seem to enhance production of IL-6 [35], another multifunctional monokine also playing a central regulatory role in infection, inflammation and tissue injury [36]. Increased IL-6 plasma levels and elevated secretion by cultured PBMC from HIV-infected patients have been reported [37].

Opportunistic infections of lung and bowel in AIDS patients may be favoured by disturbances of the mucosa-associated

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immune system. While cytokine production by peripheral blood monocytes (PBM) and alveolar macrophages from AIDS patients has been studied in some detail, corresponding studies concerning the intestinal lamina propria have not been reported so far. In the present study, we investigated the secretion of TNF-α, IL-1β and IL-6 by cultured intestinal mononuclear cells. Furthermore, we examined the cytokine secretion of monocytes/macrophages, which could easily be purified from two other compartments, PBM and alveolar macrophages. Our data show an impaired TNF-α secretion by mononuclear cells from the large bowel lamina propria of AIDS patients, while alveolar macrophages and PBM from the examined patients secreted increased amounts of TNF-α.

PATIENTS AND METHODS

Patients

Specimens were taken by colonoscopy, bronchoscopy or withdrawal of peripheral blood from patients with AIDS (WR6, CDC IVC1 or CDC IVC2 stage). From all AIDS patients who underwent colonoscopy stool samples and colon biopsies were taken and analysed for pathogenic bacteria, fungi, parasites, worm eggs and viruses. Results were obtained from 24 of the 25 patients examined. In seven of these patients, infectious agents were found. In one patient cytomegalovirus, in another *Staphylococcus aureus* was detected in the bowel. In three patients *Lamblia intestinalis* was found, in one of these *Mycobacterium avium intracellulare* and in another *Blastocystis hominis* and *Candida albicans* were detected additionally. Kryptosporidium was found in the bowel of two patients, in one of them together with *Proteus vulgaris*. In 17 patients infections of the bowel were not detected. From the AIDS patients who underwent bronchoscopy only those who had no detectable pathogens in the lavage fluid were included in this study. From these patients only, peripheral blood samples were taken for the analysis of monocytes at the time of bronchoscopy or colonoscopy. For control, colon biopsies were examined from individuals who underwent endoscopy for diagnostic reasons (e.g. exclusion of tumours), but had proven to be free of disease. For the same purpose, blood samples and bronchial lavage fluids were obtained from healthy volunteers. All specimens were taken after the patients' informed consent had been obtained.

Isolation of intestinal mononuclear cells

Lamina propria mononuclear cells (LpMNC) were isolated from endoscopically taken colon biopsies using a modification of the technique originally described by Bookman & Bull [38]. Biopsies were rinsed in RPMI 1640 medium (GIBCO-BRL, Eggenstein, Germany) and treated for 3 min with 1 mM dithiothreitol (Sigma, Heidelberg, Germany) in Ca^{2+}- and Mg^{2+}-free PBS (GIBCO-BRL). After a brief wash in PBS, biopsies were incubated at room temperature over 60 min in 1 mM EDTA (Sigma) in Ca^{2+}- and Mg^{2+}-free PBS supplemented with 10% fetal calf serum (FCS). Biopsies were washed vigorously in PBS in order to separate intraepithelial leucocytes and epithelial cells and transferred into fresh PBS. Two further washes in PBS containing 60 mg $CaCl_2$/500 ml, 55 mg $MgCl_2$/500 ml and 10% FCS were followed by treatment of the biopsies with collagenase (48 U/ml; Sigma) in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin and 50 μg/ml gentamicin for 16–18 h at 37°C in a CO_2 incubator. Aggregated tissue pieces were mechanically disrupted by pipetting and were separated from suspended lamina propria cells using a nylon sieve (105 μm pore size). LpMNC were purified by centrifugation for 10 min at 700 ***g*** in a Sigma 3K-1 centrifuge on a cushion of metrizamide (Nycomed, Oslo, Norway; dissolved in RPMI 1640 with 10% FCS). Viability of the isolated cells was usually more than 90% as tested by trypan blue exclusion. Bioptically obtained tissue (100 mg) yielded approximately 9×10^6 LpMNC.

Presence of cellular subpopulations in the isolated LpMNC was demonstrated by immunocytochemical analysis with CD45, CD68, CD22, CD4 and CD8 as primary antibodies (all from Dakopatts, Hamburg, Germany). Staining was performed with an alkaline anti-alkaline phosphatase (APAAP) kit (Dakopatts).

Preparation of alveolar macrophages

Bronchoalveolar lavage (BAL) from the lower respiratory tract was performed with a flexible bronchoscope (Olympus). Aliquots (100 ml) of sterile saline were instilled to the bronchial system by a nasally introduced bronchoscope. The lavage fluid containing suspended cells was regained by aspiration and centrifuged at 400 ***g*** for 10 min at 20°C. Cells were resuspended in supplemented RPMI 1640 culture medium. Alveolar macrophages were purified by adherence to plastic dishes for 60 min at 37°C. Plastic dishes were vigorously rinsed twice with RPMI 1640 medium at 37°C to remove non-adherent cells, and adherent cells were released with a rubber policeman. Cell viability was more than 90% as analysed by trypan blue exclusion. Purity of alveolar macrophages was more than 90% as assessed by non-specific esterase staining (Sigma) and in addition by immunoperoxidase reaction with anti-CD68 antibody (Dakopatts).

Isolation of peripheral blood monocytes

Mononuclear cells from heparinized peripheral blood were isolated by centrifugation on a lymphoprep cushion (Nycomed). Cells of the interphase were washed twice and cultured for 1 h in Petri dishes at 37°C. Petri dishes were rinsed twice with PBS in order to remove non-adherent cells, and adherent cells were released into the medium with the help of a cell scraper. The adherent cell population consisted of more than 90% PBM as determined by non-specific esterase staining (Sigma), and viability was more than 90%.

Cultivation and stimulation of mononuclear cells

PBM (5×10^4/ml), alveolar macrophages (5×10^4/ml) or LpMNC (5×10^5/ml) were cultured in 24-well plates (Nunc, Roskilde, Denmark) in RPMI 1640 medium supplemented with 10% FCS, 1% pyruvate, 100 U/ml penicillin and 100 μg/ml streptomycin (all from GIBCO-BRL). Cultures of LpMNC contained 50 μg/ml gentamicin and 2·5 μg/ml amphotericin B (GIBCO-BRL) in addition.

To determine optimal conditions for the stimulation of cytokine secretion, cells from normal donors were cultured for various time periods in the presence of lipopolysaccharide (LPS; Sigma), pokeweed mitogen (PWM; Sigma), phorbol myristate acetate (PMA; Sigma), phytohaemagglutinin (PHA-C; Sigma) in different concentrations or without stimulants.

Measurement of cytokines

The amounts of TNF-α and IL-1β in the culture supernatants were measured with specific radioimmunoassays (RIA; Medgenix, Brussels, Belgium). According to the manufacturer, in these assays all of the cytokine molecules were detected regardless of whether they were complexed or free ones. ELISAs with neutralizing antibodies were used for IFN-γ (Holland Biotechnology, Biermann, Bad, Germany) and for IL-6 (R&D Systems, Minneapolis, MN). Both assays detect only uncomplexed cytokines. All assays were performed according to the manufacturer's instructions and calibrated with cytokine standards commercially added to the kits. Standard curves were constructed and cytokine concentrations of the samples were determined by interpolation from these curves. Limits of detection were 5 pg/ml for TNF-α, 5 pg/ml for IL-1, 3·5 pg/ml for IL-6 and 0·2 U/ml for IFN-γ. TNF-α, IL-1β and IL-6 were measured in mass units (pg). In the IFN immunoassay, one unit corresponds to 100 pg pure natural human IFN-γ. Units are defined according to NIH standard number Gg23-901-530. Cytokine concentrations were always determined in triplicate cultures.

RESULTS

Cytokine secretion by intestinal mononuclear cells

Suspensions of lamina propria mononuclear cells (LpMNC) were prepared from large bowel biopsies taken during colonoscopy from AIDS patients and healthy individuals. The cellular composition of such suspensions was determined with respect to distinct surface markers (Table 1). The leucocytes in the isolated cell populations from normal controls consisted of about 23·1% CD68, 13·9% CD22, 62·6% CD3, 39·0% CD4, and 17·3% CD8 positive cells. While the percentage of CD68, CD22, CD3 and CD8 positive cells was not significantly different in LpMNC from AIDS patients and normal controls. LpMNC from AIDS patients contained a significantly reduced number of $CD4^+$ cells.

Table 1. Cellular composition of the leucocytes in the lamina propria mononuclear cell (LpMNC) preparations from normal controls and AIDS patients

Cell type	Antibody used	Percentage of individual cell types: Normal controls ($n=10$) (%)	Percentage of individual cell types: AIDS patients ($n=10$) (%)
Macrophages	CD68	23·1 ± 5·1	18·9 ± 5·5
B cells	CD22	13·9 ± 5·0	15·5 ± 7·6
T cells	CD3	62·6 ± 19·5	65·4 ± 22·7
T4 cells	CD4	39·0 ± 8·6	26·0 ± 7·6
T8 cells	CD8	17·3 ± 5·4	24·3 ± 9·0

Immunocytochemical analysis with the indicated antibodies using the APAAP method.

Cytokine secretion of cultured LpMNC from healthy individuals was analysed in three independent time course and dose response experiments with LPS, PWM, PMA, PHA-C or without stimulants. This showed that LpMNC secreted high amounts of TNF-α, IL-1β and IL-6 in the presence of 10 μg/ml PWM cultured for 48 h. LpMNC did not react to LPS (data not shown). Cytokine secretion of LpMNC from AIDS patients was compared with that of LpMNC from normal controls (Fig. 1). Basal cytokine secretion and secretion of PWM-stimulated cells from normal controls amounted to 20 pg/ml TNF-α without stimulation and 170 pg/ml TNF-α after stimulation with PWM (Fig. 1a). Constitutive TNF-α secretion of LpMNC from AIDS patients was found to be in the same range as that of cells obtained from normal controls. In contrast to the latter, the rate of TNF-α secretion of LpMNC from AIDS patients was not essentially increased after stimulation with PWM (Fig. 1a). On the other hand, secretion of IL-1β and IL-6 by LpMNC from normal controls was stimulated by PWM from 11 to 19 pg/ml and from 30 to 90 pg/ml, respectively (Fig. 1b, c). Corresponding cells from AIDS patients were found to release constitutively levels of IL-1β and IL-6 in the range of stimulated LpMNC from normal controls, i.e. 21 pg/ml IL-1β and 70 pg/ml IL-6. In these cells, the secretion of IL-1 and IL-6 could not be increased by PWM, suggesting that they were already at a maximally stimulated stage.

Since it is well known that IFN-γ may induce the generation of TNF-α, the production of IFN-γ and its influence on TNF-α secretion were studied in the *in vitro* system described here. As observed for TNF-α, IFN-γ secretion of LpMNC from normal controls and AIDS patients was comparable, at 0·6 U/ml and 0·4 U/ml, respectively (Fig. 2). While PWM increased the IFN-γ secretion by a factor of 7·3 in LpMNC from normal controls, this effect was significantly lower in corresponding cells from AIDS patients (raising factor 2·5). To test whether the diminished TNF-α secretion observed in cultured LpMNC from AIDS patients was dependent on a lack of endogenous IFN-γ, exogenous IFN-γ was added together with PWM to cultured LpMNC from AIDS patients. The presence of IFN-γ in concentrations from 1 to 1000 U/ml, however, did not increase TNF secretion by LpMNC (data not shown).

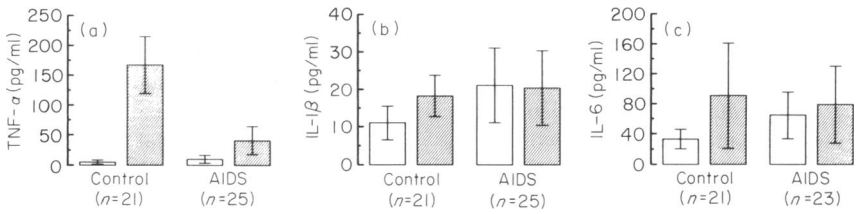

Fig. 1. Cytokine secretion by mononuclear cells of the lamina propria (LpMNC) from AIDS patients and normal donors. Cells were cultured over 48 h in the presence of pokeweed mitogen (PWM) or without stimulation. Tumour necrosis factor-alpha (TNF-α) (a), IL-1β (b), and IL-6 (c) were measured in the culture supernatants. Bars indicate s.d. □, Unstimulated; ■, 10 μg/ml PWM.

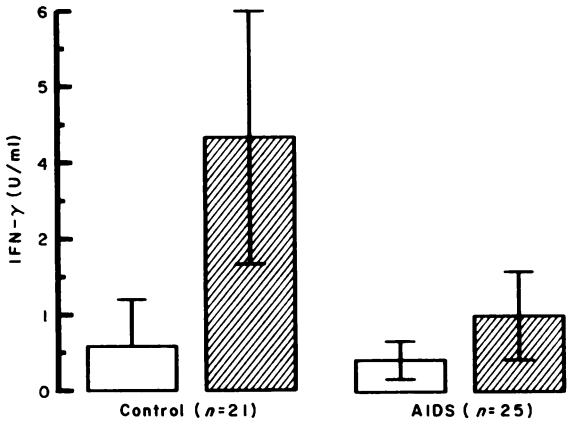

Fig. 2. Release of interferon-gamma (IFN-γ) by mononuclear cells of the lamina propria (LpMNC). Cells from AIDS patients and from normal controls were cultured over 48 h with or without stimulation by pokeweed mitogen (PWM). IFN-γ was measured in the culture supernatants. Vertical bars show s.d. □, Unstimulated; ▨, 10 μg/ml PWM.

Secretion of TNF, IL-1β and IL-6 by peripheral blood monocytes

Monocytes were isolated from the peripheral blood of normal controls and AIDS patients, and cytokine secretion was measured. In three independent time course and dose response experiments with LPS, PWM, PMA or PHA-C, it was observed that secretion of TNF-α, IL-1β and IL-6 by PBM was effectively stimulated by treatment with 30 μg/ml LPS or 25 μg/ml PWM for 24 h (data not shown). PBM from normal controls secreted 730 pg/ml TNF-α spontaneously and 1500 pg/ml after stimulation with PWM or LPS (Fig. 3a). Corresponding cells from AIDS patients constitutively secreted higher TNF-α levels, i.e. 2400 pg/ml. In contrast to LpMNC, PBM from AIDS patients could be stimulated to increased TNF-α secretion by PWM or LPS (4000 pg/ml).

A similar pattern of secretion was observed for IL-1β and IL-6 (Fig. 3b, c). Thus, culturing PBM from normal controls in the presence of PWM or LPS, concentrations were increased by a factor of 5·3 for IL-1β and by a factor of 4·2 or 5·7, respectively, for IL-6. PBM from AIDS patients secreted higher basal cytokine amounts, i.e. 1100 pg/ml IL-1β and 2400 pg/ml IL-6. Although the secretion rate could be further increased by stimulation with PWM or LPS to around 2000 pg/ml for IL-1β and to around 4000 pg/ml for IL-6, the degree of increase was considerably lower (1·8 and 1·7, respectively) than that seen after stimulation of PBM from normals (see above).

Secretion of cytokines by alveolar macrophages

Release of TNF-α, IL-1β and IL-6 by alveolar macrophages was measured (Fig. 4). In three independent time course and dose response experiments with LPS, PWM, PMA or PHA-C, highest stimulation of alveolar macrophages was obtained using 30 μg/ml LPS or 20 μg/ml PWM for 24 h (data not shown). Unstimulated alveolar macrophages from normal controls produced 1200 pg/ml TNF-α compared with 2000 pg/ml or 2400 pg/ml after stimulation by PWM or LPS, respectively (Fig. 4a). As PBM, alveolar macrophages from AIDS patients showed a higher constitutive TNF-α production (2700 pg/ml) and were stimulated to enhanced secretion by PWM (6000 pg/ml) or LPS (7500 pg/ml).

For IL-1β and IL-6 a similar secretion pattern was found (Fig. 4c, d). In cultures of alveolar macrophages from normal controls, concentrations of IL-1β were increased by a factor of 4·6 using PWM and by a factor of 7·3 using LPS. Secretion of IL-6 was increased 26-fold by PWM and 33-fold by LPS. Using alveolar macrophages from AIDS patients, higher basal con-

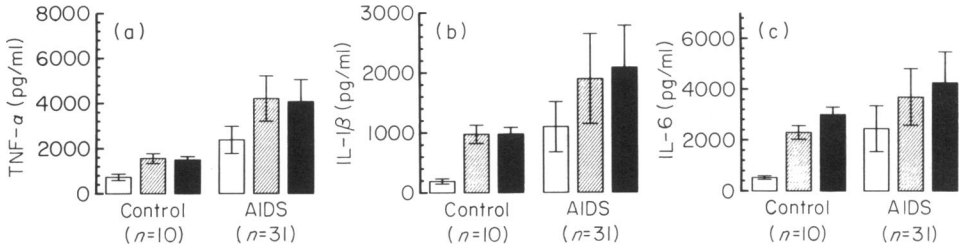

Fig. 3. Secretion of tumour necrosis factor-alpha (TNF-α) (a), IL-1β (b) and IL-6 (c) by peripheral blood monocytes from AIDS patients in comparison with secretion by corresponding cells from normal donors. Cells were cultured over 24 h in the presence of pokeweed mitogen (PWM), lipopolysaccharide (LPS) or without stimulation, and the cytokines were measured in the culture supernatants. Vertical bars indicate s.d. □, Unstimulated; ■, 30 μg/ml LPS; ▨, 25 μg/ml PWM.

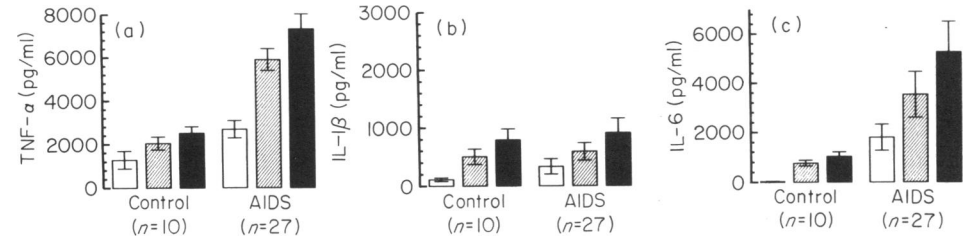

Fig. 4. Concentrations of tumour necrosis factor-alpha (TNF-α) (a), IL-1β (b) and IL-6 (c) in cultures of alveolar macrophages from AIDS patients and from normal controls. Cells were cultured over 24 h in the presence of pokeweed mitogen (PWM), lipopolysaccharide (LPS) or without stimulation. Vertical bars indicate s.d. □, Unstimulated; ■, 30 μg/ml LPS; ▨, 20 μg/ml PWM.

centrations were found, and cytokine release could be further enhanced by PWM or LPS. The degree of increase, however, was lower (for IL-1β 1·8- or 2·7-fold, for IL-6 1·9- or 2·9-fold in the presence of PWM or LPS, respectively) than that observed with alveolar macrophages from healthy controls (see above).

DISCUSSION

Here we present data concerning the secretion of TNF-α, IL-1β and IL-6 by LpMNC from AIDS patients and normal controls. Constitutive secretion of TNF-α by LpMNC was low. This may be part of a physiological down-regulation of a number of macrophage functions, such as the reduced production of reactive oxygen intermediates in the lamina propria [39]. Among several agents tested, PWM stimulation of normal LpMNC resulted in increased release of TNF, IL-1β and IL-6 in a time- and dose-dependent manner (data not shown). By contrast, LpMNC did not react to LPS, probably because LpMNC were permanently exposed to LPS *in vivo*. Using LpMNC from AIDS patients, PWM treatment resulted only in a small increase in TNF-α release, whereas a clearly enhanced TNF-α secretion was seen when normal LpMNC were stimulated by PWM. In contrast, enhanced release of IL-1β and IL-6 was observed with stimulated LpMNC even from AIDS patients. Macrophages are considered to be the main source for TNF-α [13], and here we have shown that the number of macrophages was not reduced in the lamina propria of the large bowel in AIDS patients. This, indeed, could lead to the suggestion that lamina propria macrophages from AIDS patients might have impaired potency for TNF-α secretion.

In order to examine whether the aberrant behaviour in cytokine secretion of LpMNC from HIV-infected individuals reflects a basic overall defect of the monocyte/macrophage system in these patients, or a defect restricted to their gut-associated phagocytes, or whether it is inherent in the techniques used in these experiments, purified monocytes/macrophages from other compartments were investigated for cytokine release by the use of similar techniques. LPS was proved to be a powerful inducer of cytokines in normal alveolar macrophages and was as effective as PWM for cytokine induction in normal PBM. Alveolar macrophages and PBM from AIDS patients secreted increased amounts of TNF-α, IL-1β and IL-6 both with and without stimulation compared with corresponding cells from normal controls. The increased production of TNF-α by PBM and alveolar macrophages from AIDS patients described here accords well with observations by others [4,5,8–10]. Similarly, our data are in accordance with the findings of Breen *et al.* [37] that spontaneous monocyte-mediated IL-6 production is also increased in HIV-infected patients. While we and some authors [4,11,12] have found fairly elevated IL-1 secretion by peripheral blood cells from HIV-infected patients, others failed to confirm this phenomenon [3]. The reason for these discrepancies may be the use of different cell populations (monocytes *versus* mononuclear cells) and culture conditions. Moreover, the IL-1 bioassay used in some studies may be influenced by an IL-1 inhibitor [11]. In our study, however, we applied an immunoassay with polyclonal antiserum against IL-1 detecting all cytokine molecules, i.e. free and complexed ones.

The differences in TNF-α secretion and the diverging response to PWM and LPS observed in LpMNC compared with alveolar macrophages and PBM from AIDS patients are probably due to the fact that alveolar macrophages and PBM were highly purified cell populations, whereas for technical reasons the LpMNC were highly contaminated with lymphocytes and contained only about 20% macrophages. Thus, the presence of lymphocytes in the LpMNC population of AIDS patients may have affected cytokine production directly via lymphokines and indirectly in potential interactions between the different cell types present in the cultures. Therefore, a direct comparison between the three cell populations is somewhat problematic. It is also possible that the functional heterogeneity is attributed to differences in the activation state of the cells or to various anatomical sites of cellular origin (intestine, peripheral blood, lung), arguing for different monocyte/macrophage populations in various microenvironments. Furthermore, the separative procedure for LpMNC could influence the functional properties of the cells obtained. The described differences in TNF-α production between controls and AIDS patients might reflect differing sensitivity of the LpMNC to the isolation method used but indirectly represent differences in the behaviour of the cells.

HIV infection of macrophages themselves could affect TNF-α production. There is evidence that cells of the monocyte/macrophage lineage may harbour HIV and thus may play an important role in the dissemination of HIV infection within the body. Infected monocytes/macrophages seem to be relatively resistant to the cytopathic effect of the virus [40–42]. It has been reported that cells of the monocytic cell line THP-1 [43], PBMC [44] and monocytes [45] exposed to reproductive or inactivated HIV could produce IL-1β, IL-6 and TNF-α, where obviously the HIV-1 protein gp120 has the intrinsic capacity to stimulate monokine secretion [46]. On the other hand, several cytokines (including IL-1 and IL-6) have demonstrated up-regulatory effects on HIV production in mononuclear phagocytes [47]. TNF-α appears to up-regulate HIV expression by activation of nuclear factor kappa B, which binds to specific consensus sequences present in the HIV long terminal repeat [48–51]. Thus, HIV may indirectly augment its own reproduction. In contrast to the elevated TNF-α secretion by PBM and alveolar macrophages, our data show that intestinal mononuclear cells from AIDS patients have an impaired TNF-α secretion. TNF-α production by these cells might be influenced by coinfection with other viruses or bacteria. It has been reported that human cytomegalovirus-stimulated PBMC could induce HIV-1 replication via a TNF-α-mediated mechanism [52], and for alveolar macrophages, a markedly increased spontaneous TNF-α production has been described in HIV$^+$ patients with active *Pneumocystis carinii* pneumonia compared with patients without the infection [8]. However, from AIDS patients, whose intestinal mononuclear cells were examined in the present study, 17 patients had no detectable bowel infection, and in seven patients various infectious agents were found.

Hypothetically, the low TNF-α secretion by LpMNC from AIDS patients might be due to the reduced number and functional impairment of CD4$^+$ cells resulting in a reduced production of macrophage-activating T cell-derived cytokines. The number of CD4$^+$ cells in the lamina propria was, as expected, found diminished in this study and by others [53], and IFN-γ was found reduced in the supernatants of LpMNC from AIDS patients. However, addition of exogenous IFN-γ to LpMNC from HIV-infected individuals did not induce TNF-α secretion. A general impairment of macrophage functions in the

lamina propria of HIV-infected patients, however, seems to be unlikely, since our data did not reveal a reduced secretion of the monokines IL-1β or IL-6.

In recent years, it has been suggested that endogenous mediators are essential elements in the pathogenesis of cachexia which is observed in chronic infectious and neoplastic diseases. The anorexia observed in laboratory animals after treatment with TNF-α [54–56], the catabolic effects of TNF-α and its influence on lipid metabolisms [57–59] suggest that this cytokine may take part in the complex metabolic changes resulting in cachexia. High TNF-α serum levels and high production of TNF-α by monocytes/macrophages are hints that TNF-α/cachectin might be a central mediator of wasting observed in AIDS.

Lack of TNF-α in the lamina propria of the large bowel could result in reduced adhesion of circulating cells to endothelial cells which is critical for initiation of inflammation at the site of injury and which can be increased by TNF-α [60]. Furthermore, the external transport of locally produced IgA might be impaired, since TNF-α can up-regulate the intracellular pool and epithelial membrane expression of the secretory component [61]. The lack of this cytokine could be one contributing factor along with other major components such as IgA, resulting in reduced defence mechanisms of the mucosal immune system in the bowel of patients with full-blown AIDS.

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